HIGHLY OXIDIZED γ-LACTAM-CONTAINING NATURAL PRODUCTS: TOTAL SYNTHESIS AND BIOLOGICAL EVALUATION

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Abstract – γ-Lactam is a ubiquitous structure found in the natural products. A number of highly oxidized γ-lactam-containing natural products are produced by various fungi. These compounds often show a wide range of biological activities because their multiple internal reaction sites, which arise from the high oxidation state of the compounds, can react with biological nucleophiles. Due to their high reactivity and dense functionality, total syntheses of these molecules require strict control of the inherent reactivity and the appropriate design of synthetic intermediates. This review focuses on the recent total syntheses of some highly oxidized γ-lactam-containing natural products, including fused bicyclic (epolactaene, NG-391, lucilactaene, L-755,807), spirocyclic (azaspirene, pseudotin A, E, and F2, cephalimysin A–C, FD-838, and berkeleyamide D), and tricyclic (rubrobramide and talaramide A) skeletons, and on the structure-activity relationship studies of related molecules.
INTRODUCTION

Lactam-containing compounds are widely used in various areas of contemporary science owing to their useful chemical and physical properties. For example, ε-lactams are a common fiber feedstock\textsuperscript{1,3} and β-lactam is a partial structure in a wide range of antibacterial agents, including penicillin and cephalosporin.\textsuperscript{4,5} Functionalized γ-lactam structures are frequently found in natural products. In particular, highly oxidized γ-lactam-containing molecules show a diverse range biological activities, such as anticancer, antibacterial, neuroprotective, and anti-Alzheimer activities. The attractive chemical structures
of these compounds and their promising biological activity have made them synthetic targets in chemistry research worldwide. Several total syntheses have been achieved, particularly by Japanese synthetic organic chemists. Furthermore, given the potential of these natural products as pharmaceutical agents, structure-activity relationship (SAR) studies have also been conducted as part of drug discovery research. In this review, we summarize the efficient total syntheses of some natural products containing a highly oxidized γ-lactam structure and SAR studies from the last two decades.

2. SYNTHESIS OF γ-LACTAM-CONTAINING NATURAL PRODUCTS WITH FUSED BICYCLIC RING SYSTEMS

Some natural products, including epolactaene (1),6 NG-391 (2),7 lucilactaene (3),8 and L-755,807 (4),9 that contain a fused bicyclic highly oxidized γ-lactam connected to a long conjugated lipophilic side chain exhibit promising bioactivity against cancer and Alzheimer’s disease (Figure 1). However, the absolute stereostructures of these molecules were not determined when they were isolated, and thus they have required structural determination by chemical synthesis, making them valuable synthetic targets.

Epolactaene (1) was first isolated as a diastereomeric mixture at the hemiaminal position (ca. 5:1 ratio) from the culture broth of Penicillium sp. BM 1689-P by Osada et al.6 Epolactaene (1) consists of a distinctive epoxy-γ-lactam and a side chain containing a conjugated (E,E,E)-triene and (E)-α,β-unsaturated ketone, and the compound induces neurite outgrowth and arrests the cell cycle at the G0/G1 phase in the SH-SY5Y human neuroblastoma cell line.10 Subsequently, 1 has been found to inhibit the growth of human T-lymphoma Jurkat cells,11,12 inhibit mammalian DNA polymerases and human DNA topoisomerase II,13,14 induce apoptosis in the BALL-1 human leukemia B-cell line,15,16 and have anti-inflammatory activity.14,17 In addition, epolactaene has several electrophilic reactive sites, such as the α,β-unsaturated ketone, epoxide, and hemiaminal carbon, that can react with biological nucleophiles, and thus, it may exhibit other attractive bioactivities. Due to its interesting chemical structure and promising biological activity, several research groups have reported total syntheses of 1 via various synthetic strategies.

NG-391 (2) was isolated from Fusarium sp. TF-0452,2 and lucilactaene (3) was isolated from Fusarium sp. RK 97-94 together with NG-391, and 3 exhibited an optical rotation of 0 in two different solvents.3 Compounds 2 and 3 were expected to show bioactivities related to those of epolactaene, which exhibits a variety of biological properties, owing to structural similarities among these compounds. Indeed, 2 shows neurotrophic activity and affects neurite outgrowth, and 3 inhibits the cell cycle in p53-transfected cancer cells.3

L-755,807 (4), isolated from an endophytic fungus, Microsphaeropsis sp., is a bradykinin B2 antagonist.4 The compound contains a labile tetraene side chain with two stereocenters and a characteristic
epoxy-γ-lactam, similar to epolactaene and NG-391. Therefore, L-755,807 (4) is also expected to show neurite outgrowth activity.

Figure 1. γ-Lactam-containing Natural Products with Fused Bicyclic Ring Systems

2-1-1. Kogen’s Total Syntheses of Epolactaene

Kogen and co-workers reported the first total synthesis of epolactaene (1) by a highly convergent synthetic strategy (Scheme 1). They developed a protecting group-controlled diastereoselective aldol reaction to synthesize both enantiomers of epolactaene from D-(+)-lactic acid (5) as a common starting material. Thus, aldehyde 6, which was prepared from 5, underwent the aldol reaction with di-tert-butyl malonate. The bulky trityl protecting group on the hydroxy group provided desirable aldol adduct 7 with good syn selectivity. Aldol adduct 7 was transformed to epoxide 9 via removal and reintroduction of the protecting group, iodination of the methine proton, and TMS cleavage and spontaneous epoxidation. After further transformation of epoxide 9 into diamide 12 via lactones 10 and 11, the vinyllithium species generated from 13 was reacted with Weinreb amide 12 to afford an (E)-α,β-unsaturated ketone. Wittig reaction of aldehyde 14 gave conjugated (E,E,E)-triene 16 with good stereoselectivity. They completed the total synthesis of (+)-epolactaene (1) after desilylation and spontaneous oxidative cyclization. In addition, substrate 17 with a small benzyl protecting group preferentially afforded the anti-aldol adduct, 18. (−)-Epolactaene (1) could be easily accessed from 18 via a similar synthetic scheme to the one used for the (+)-enantiomer.
In 2014, Kogen’s group reported its second-generation formal synthesis of epolactaene (1) (Scheme 2). They realized that the first-generation synthesis was still unsatisfactory because of the moderate stereoselectivity and yield, especially in the key aldol reaction. Additionally, to circumvent the problematic synthetic sequence, they planned an alternative strategy, using a highly diastereoselective epoxidation of an olefinic diol. Hence, TBDPS-protected aldehyde 20, prepared from L-lactic acid (19), was reacted with Horner–Wadsworth–Emmons (HWE) reagent 21 to give bromoacrylate derivative 22 with complete stereoselectivity. Based on their synthetic plan, bromoacrylate 22 was converted to...
olefinic diol 23, which was subjected to epoxidation with VO(acac)$_2$ and tert-butyl hydroperoxide (TBHP) to deliver desired epoxide 24 in excellent yield and diastereoselectivity. Diol epoxide 24 could be converted to lactone 27, a known synthetic intermediate, via unstable dicarboxylic acid 25 and diamide 26, completing the formal synthesis of (+)-epolactaene (1).

![Scheme 2. Kogen’s Second-Generation Total Synthesis of Epolactaene](image)

2-1-2-1. Hayashi’s Total Synthesis of Epolactaene

Hayashi’s group published the total synthesis of epolactaene (1) almost simultaneously with the first report from Kogen’s group (Scheme 3). In their synthetic route, (E,E,E)-triene ester portion of epolactaene was efficiently constructed in the beginning of the synthesis. Thus, (E,E,E)-dienecarboxylate 29, derived from tetrahydropyran-2-ol (28), was initially reacted with acetaldehyde via kinetic deprotonation to give α-adduct 30 with high yield and regioselectivity, and (E,E,E)-triene 31 was created by subsequent dehydration and isomerization. Then, aldehyde 32 underwent HWE olefination using β-ketothioester diethyl phosphonate derivative 33 to provide an γ,δ-unsaturated β-ketothioester. The key Knoevenagel condensation between amide 34 and (R)-2-(tert-butyldimethylsiloxymethoxy)propanal (35) gave desired products 36, albeit in low yield. Nucleophilic epoxidation with TrOOLi yielded epoxide 37 in moderate stereoselectivity. The total synthesis of epolactaene (1) was completed by deprotection, separation of the epoxide isomers, and oxidation of the resultant alcohol.
To overcome the drawbacks in the Knoevenagel condensation and epoxidation steps, Hayashi’s group developed a modified approach to epolactaene (1) by optimizing the substrate in each step (Scheme 4).\textsuperscript{24} Using β-ketonitrile 40 instead of β-ketoamide 34 as the substrate in the Knoevenagel condensation improved the stereoselectivity and yield considerably. They assumed that the improvement was due to the cyano group decreasing steric repulsion and the increased thermodynamic stability of the product. Additionally, incorporating the bulkier triethylsilyl group dramatically increased the stereoselectivity in the epoxidation step (42→43). They completed the improved total synthesis by the appropriate transformation to epolactaene from epoxide 43.
Scheme 4. Hayashi’s Second-Generation Total Synthesis of Epolactaene

2-1-2-2. Hayashi’s SAR Studies of Epolactaene

After completing the total synthesis of epolactaene (1), Hayashi’s and Osada’s groups launched joint broad biological studies to investigate the inhibitory activity against the growth of SH-SY5Y and Jurkat cells, the target protein of epolactaene, and the interaction mode of the compounds with the protein.\textsuperscript{11} Based on their synthetic scheme for epolactaene (1), they prepared several epolactaene analogs, including side-chain analog 48 without a γ-lactam, 49–52 with simplified lipophilic side chains, and biotin analog 53. They biologically evaluated the inhibition ability of the synthetic compounds against the growth of SH-SY5Y and Jurkat cells (Table 1). The results suggested that the γ-lactam, α,β-saturated ketone, and length of the alkyl side chain were crucial for the potent biological activities, whereas the triene and ester moieties may not be essential. Furthermore, biotin-tagged analog 53 was used as a chemical probe to identify human heat shock protein (Hsp) 60 as the binding target of epolactaene (1), which interacted with Cys\textsuperscript{442} of Hsp60 via the terminal Michael acceptor moiety in the side chain.\textsuperscript{11,12}
Table 1. IC\textsubscript{50} Acting Against SH-SY5Y and Jurkat Cell Viability by Epolactaene Derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC\textsubscript{50} (μM)</th>
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<td>(+)-epolactaene (1)</td>
<td>SH-SY5Y cells</td>
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<td>13.0</td>
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<tr>
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</table>

2-1-3-1. Kobayashi’s Total Synthesis of Epolactaene

Kobayashi’s group also reported the total synthesis of epolactaene (1) (Scheme 5).\textsuperscript{25} Their convergent synthetic strategy divided 1 into the epoxy-γ-lactam and side-chain segments. A Wittig reaction between aldehyde 54 and phosphonium salt 55 was followed by stereoselective carbometallation with \( \text{Cp}_2\text{TiCl}_2-\text{Me}_3\text{Al} \) and subsequent iodination to afford dienyl iodide 57, which was connected with vinylstannane 58 by Stille coupling to construct the triene portion of the epolactaene side chain. Side-chain segment 62 was formed by Wittig homologation, and α-trimethylsilyl epoxylactone 64 was prepared as the epoxy-γ-lactam segment. To couple synthetic segments 62 and 64, they used the distinctive nucleophilic addition of an oxiranyl anion, generated from 64 with catalytic TBAF, to aldehyde 62 to obtain a coupling product. This was the first report of using an oxiranyl anion generated from epoxylactone.\textsuperscript{26} Finally, the lactone moiety in 65 was converted to a lactam to complete the total synthesis of epolactaene (1).
Kobayashi’s and Ikekita’s groups investigated the action of epolactaene and its derivatives on DNA metabolic enzymes during their studies of biologically active molecules affecting DNA replication, repair, and recombination.\textsuperscript{13-17} Thus, they prepared and biologically evaluated various epolactaene analogs. Initial screening of the compounds for inhibition of mammalian DNA polymerases\textsuperscript{14} and human DNA topoisomerase II\textsuperscript{13} indicated the importance of the \( \alpha,\beta \)-epoxy-\( \gamma \)-lactam and the long alkyl chain, implying that hydrophobicity was crucial for the activities. This hypothesis was supported by the correlation of the activities with octanol/water partition coefficient (ClogP) values (Table 2).\textsuperscript{14} They evaluated the apoptosis-inducing effect of the synthetic analogs on BALL-1 cells,\textsuperscript{16} and the results suggested that the \( \alpha \)-acyl-\( \alpha,\beta \)-epoxy-\( \gamma \)-lactam structure, along with the long side chain with high ClogP values, were required for the activity, which was attributed to permeation across the cell membrane.\textsuperscript{15,16} However, the stereochemistry of the epoxide had no effect on the activity.

Scheme 5. Kobayashi’s Total Synthesis of Epolactaene

2-1-3-2. Kobayashi’s SAR Studies of Epolactaene

Kobayashi’s and Ikeda’s groups investigated the action of epolactaene and its derivatives on DNA metabolic enzymes during their studies of biologically active molecules affecting DNA replication, repair, and recombination.\textsuperscript{13-17} Thus, they prepared and biologically evaluated various epolactaene analogs. Initial screening of the compounds for inhibition of mammalian DNA polymerases\textsuperscript{14} and human DNA topoisomerase II\textsuperscript{13} indicated the importance of the \( \alpha,\beta \)-epoxy-\( \gamma \)-lactam and the long alkyl chain, implying that hydrophobicity was crucial for the activities. This hypothesis was supported by the correlation of the activities with octanol/water partition coefficient (ClogP) values (Table 2).\textsuperscript{14} They evaluated the apoptosis-inducing effect of the synthetic analogs on BALL-1 cells,\textsuperscript{16} and the results suggested that the \( \alpha \)-acyl-\( \alpha,\beta \)-epoxy-\( \gamma \)-lactam structure, along with the long side chain with high ClogP values, were required for the activity, which was attributed to permeation across the cell membrane.\textsuperscript{15,16} However, the stereochemistry of the epoxide had no effect on the activity.
Table 2. Biological Evaluations and ClogP Values of Epolactaene and Its Derivatives

\[
\text{\textbf{1a-e}} \quad \text{\textbf{66a-d}} \quad \text{\textbf{65a-d}} \quad \text{\textbf{67a-d}} \quad \text{\textbf{68a-d}} \\
\text{a: } R^1 = \text{MeCO}_2 \quad \text{b: } R^1 = n-C_{11}H_{23} \quad \text{c: } R^1 = \text{n-C}_3H_{11} \quad \text{d: } R^1 = n-C_{17}H_{35} \quad \text{e: } R^1 = n-C_{12}H_{25} \quad \text{f: } R^2 = n-C_6H_{13}
\]

\[
\text{\textbf{75}} \xrightarrow{1) \text{LDA, THF}} \text{Me(\text{CH}_2)_{11}OTf} \xrightarrow{2) \text{HF aq., MeCN}} \text{\textbf{76}} \xrightarrow{1) \text{NH}_3 \text{aq., MeOH}} \text{\textbf{77}} \\
\text{1) } \text{LiOH, THF/H}_2\text{O}
\]

\[
\text{\textbf{78}} \xrightarrow{1) \text{TBHP, Ti(Oi-Pr)}_4, \text{MS3A, CH}_2\text{Cl}_2} \text{\textbf{79}} \xrightarrow{1) \text{CH}_2(\text{CO}_2\text{Me})_2, \text{Cs}_2\text{CO}_3, 18\text{-crown-6, THF}} \text{\textbf{80}} \xrightarrow{1) n\text{-BuLi, THF}} \text{\textbf{81}} \\
\text{2) NH}_3 \text{aq., MeOH} \quad \text{3) TBSCl, imidazole, DMF}
\]

\[
\text{\textbf{82}} \xrightarrow{1) \text{NH}(\text{OMe})\text{Me-HCl, BOP, i-Pr}_2\text{NEt, CH}_2\text{Cl}_2} \text{\textbf{83}} \xrightarrow{1) \text{NaOH aq., MeOH}} \text{\textbf{84}} \xrightarrow{1) \text{NH}_3 \text{aq., MeOH}} \text{\textbf{85}} \\
\text{2) DMP, CH}_2\text{Cl}_2
\]

1 ) T BH P, Ti (O i -Pr) _4, MS 3 A, CH _2 Cl 2
2 ) N sC l, N Et _3, CH _2 Cl 2
1 ) CH _2( CO _2 Me) _2, Cs _2 CO _3, 18 -crown -6, THF
2 ) N sC l, N Et _3, CH _2 Cl 2
1 ) NH(OMe)Me-HCl, BOP, i-Pr2NEt, CH2Cl2
2 ) NH3 aq., MeOH
3 ) TBSCl, imidazole, DMF
1 ) NH(OMe)Me-HCl, BOP, i-Pr2NEt, CH2Cl2
2 ) NaOH aq., MeOH
Me(\text{CH}_2)_{15}\text{NHCH}_3\text{BO P, i-Pr}_2\text{NEt, CH}_2\text{Cl}_2
1 ) NH3 aq., MeOH
2 ) DMP, CH2Cl2
1 ) NH3 aq., MeOH
2 ) DMP, CH2Cl2
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⁹ Not tested. ¹¹ Not determined.

To clarify the reaction site and effects on the target protein, epolactaene and its analogs were reacted with N-acetylcysteine methyl ester 86, which mimicked cysteines in the protein (Scheme 6). The reaction proceeded at the α-position of the epoxy-γ-lactam core structure to give corresponding intermediate 88, which led to the formation of a disulfide bond. Thus, they concluded that epolactaene and its analogs induced intramolecular or intermolecular disulfide formation with cysteines in the protein. ²⁸
2-1-4. Negishi’s Total Synthesis of Epolactaene

In 2006, Negishi’s group accomplished the total synthesis of epolactaene (1) by a Pd-catalyzed homoallyl-alkenyl coupling and a late-stage coupling between the epoxy-γ-lactam and side-chain segments inspired by Kobayashi’s procedure (Scheme 7).29 Side-chain segment 62 was synthesized from propargyl alcohol by two types of carboalumination using Cp₂TiCl₂-Me₃Al or Cp₂TiCl₂-DIBAL-H and Pd-catalyzed cross-coupling reactions with organozinc reagents, whereas epoxy-γ-lactam segment 64 was constructed by a Ti-catalyzed trans-hydromagnesation followed by CO₂ fixation, acid-catalyzed lactonization, and nucleophilic epoxidation. Completion of the total synthesis of epolactaene (1) was accomplished by following Kobayashi’s protocol.25
Scheme 7. Negishi’s Total Synthesis of Epolactaene

2-2. Hayashi’s Total Synthesis of Lucilactaene from NG-391

Hayashi et al. completed the asymmetric total synthesis of lucilactaene (3) via NG-391 (2) by modifying their synthetic scheme for epolactaene and using a new protecting group (Scheme 8).

To prepare NG-391 (2), (E,E,E)-alcohol 97, a synthetic intermediate for epolactaene, was first oxidized to (E,E,E,E)-aldehyde 98 with IBX (Scheme 8). According to the synthetic protocol for epolactaene, they synthesized NG-391 (2) through the condensation of β-ketonitrile 99 and (S)-malic acid-derived aldehyde 100.
Next, they investigated the synthesis of lucilactaene (3) from NG-391 (2) (Scheme 9). NG-391 (2) was initially converted to β-methoxide 103 as a single isomer. After Boc protection, the epoxide portion in 104 was reduced with SmI$_2$, and α,β-unsaturated ketoamide 105 was treated with TFA in CH$_2$Cl$_2$/H$_2$O to form lucilactaene (3). The optical rotation of synthetic 3 was 0, identical to that of natural lucilactaene. However, compound 106, which was obtained in the final step along with 3, had a value of [α]$_D$ +36.6 (c 0.17, MeOH). Therefore, they hypothesized that synthetic 3 was racemized during the final step by treatment with acid. Hence, to obtain optically pure lucilactaene, they decided to develop an alternative synthetic route that would require a new protecting group that was easily removable without an acid.

Synthetic NG-391 (2) was transformed to phenylselenylethyl ether 107 by using 2-(phenylselenyl)ethan-1-ol (Scheme 9). Boc protection, reduction of 108 with SmI$_2$, and Boc deprotection of 109 gave requisite bicyclic lactam 110, which was oxidized to selenoxide 111 with dimethyldioxirane (DMDO). DABCO-promoted β-elimination gave vinyl ether 112, and final oxidative treatment with DMDO furnished lucilactaene (3). Thus, phenylselenylethyl ether performed well as a new protecting group to give optically pure lucilactaene ([α]$_D$ +39.5 (c 0.10, MeOH)).
In 2016, Kobayashi and Kogen achieved the first total synthesis of L-755,807 (4) and three stereoisomers to establish the relative and absolute configurations of the natural product (Scheme 10).\textsuperscript{32-34} The key reactions were a diastereoselective Darzens condensation\textsuperscript{35} to synthesize the epoxy-\(\gamma\)-lactam segment and an HWE reaction\textsuperscript{22} to construct the trisubstituted olefin in the side chain.

Darzens condensation of the aldehyde prepared from D-valine (113) with tert-butyl bromomalonate gave epoxide 115 with high diastereoselectivity, and 115 was immediately converted to phosphonate 118 as the epoxy-\(\gamma\)-lactam segment via lactone 116 and diamide 117.

Scheme 9. Hayashi’s Total Synthesis of Lucilactaene

2-3-1. Kobayashi and Kogen’s Total Synthesis of L-755,807

In 2016, Kobayashi and Kogen achieved the first total synthesis of L-755,807 (4) and three stereoisomers to establish the relative and absolute configurations of the natural product (Scheme 10).\textsuperscript{32-34} The key reactions were a diastereoselective Darzens condensation\textsuperscript{35} to synthesize the epoxy-\(\gamma\)-lactam segment and an HWE reaction\textsuperscript{22} to construct the trisubstituted olefin in the side chain.

Darzens condensation of the aldehyde prepared from D-valine (113) with tert-butyl bromomalonate gave epoxide 115 with high diastereoselectivity, and 115 was immediately converted to phosphonate 118 as the epoxy-\(\gamma\)-lactam segment via lactone 116 and diamide 117.
For the side-chain segment, known alcohol 119 was converted to α,β-unsaturated ester 120 with high stereoselectivity by an HWE reaction, and then the ester group was reduced to a methyl group to afford vinyl bromide 121. The Suzuki–Miyaura coupling of vinyl bromide 121 with known boronic acid 122 and subsequent oxidation with MnO2 yielded side-chain segment 123. Triene aldehyde 123 was coupled with 118 to deliver tetraene ketone 124, and then desilylation and AZADOL oxidation furnished L-755,807 (4) and its isomer 125 at the hemiaminal position, establishing the relative and absolute configurations of natural 4.

Scheme 10. Kobayashi and Koge's Total Synthesis of L-755,807
2-3-2. Kobayashi and Kogen’s SAR Studies of L-755,807
After completion of the synthesis of L-755,807 (4), Kobayashi and Kogen evaluated the inhibitory activity of the synthetic compounds against amyloid-β aggregation for drug discovery in Alzheimer’s disease (Table 3). Synthetic L-755,807 and its stereoisomers showed potent inhibitory activity. Isomer 127, which had the opposite configurations to the natural product at C5, C18, and C20, exhibited the most potent activity.³³,³⁴ Toward pharmacophore identification, they also prepared and biologically evaluated analogs 128–132, which revealed that an epoxy-γ-lactam moiety was not essential for the activity, whereas the amphiphilicity of the compounds, as shown by 131 and 132, was crucial for potent inhibitory activity.³⁸

Table 3. Aβ Aggregation Inhibitory Activity of L-755,807 and Its Derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>Aβ aggregation inhibitory activity, IC₅₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 (L-755,807)</td>
<td>21</td>
</tr>
<tr>
<td>125</td>
<td>17</td>
</tr>
<tr>
<td>126</td>
<td>12</td>
</tr>
<tr>
<td>127</td>
<td>4</td>
</tr>
<tr>
<td>128</td>
<td>601</td>
</tr>
</tbody>
</table>
3. SYNTHESIS OF γ-LACTAM-CONTAINING NATURAL PRODUCTS WITH SPIRO AND TRICYCLIC RING SYSTEMS

Many highly oxidized γ-lactam containing compounds with a 1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione core have been isolated as fungal metabolites and also possess various attractive biological activities (Figure 2). The syntheses and biosynthetic pathways of these compounds have been studied extensively and are summarized in the review by Jo and Han. In this section, we introduce compounds for which total synthesis has been achieved or is being investigated.

Azaspirene (133) was isolated from the fungus *Neosartorya* sp. by the Osada and Kakeya groups and was identified as a novel angiogenesis inhibitor. They found that natural product 133 inhibited endothelial migration induced by avascular endothelial growth factor (ED_{100} = 27.1 μM), and the *in vivo* antiangiogenic activity was confirmed by a tumor neoangiogenesis assay and a chicken chorioallantoic membrane (CAM) assay. Additionally, they showed that 133 specifically blocks vascular endothelial growth factor-induced phosphorylation of Rik1-associated factor. Based on these results, natural product 133 is widely expected to have clinical utility for the treatment of cancer, diabetic retinopathy, rheumatoid arthritis, and related disease.

Pseurotins A (134) and E (135) were isolated from a culture broth of *Pseudeurotium ovalis* STOLK together with pseurotin analogues (pseurotins B–D). The structure of pseurotin A (134) was determined by single-crystal X-ray analysis of its 12,13-dibromo derivative. Pseurotin A (134) has diverse inhibitory activities against chitin synthase (IC_{50} = 81 mM), MAO (42.7% inhibitory activity at 0.1 mg/mL), and IgE production *in vitro* (IC_{50} = 3.6 mM; details are given in Section 3-4-2). Moreover, pseurotin A (134) acts on cell types associated with neural diseases or cancer. The compound 134 shows neuritogenic activity in rat pheochromocytoma PC12 cells, cytotoxicity against A2780 human ovarian carcinoma cells (IC_{50} = 12 μg/mL), apomorphine antagonist activity, and cytotoxicity against the HL-60 cell line (IC_{50} = 67.0 mM). Although the biological activity of pseurotin A has been studied extensively, that of pseurotin E (135) has not. Pseurotin F_2 (136) was isolated from *Aspergillus fumigatus* DSM 6598 together with pseurotin F_1. Natural product 136 exhibited inhibitory activity against chitin synthase (IC_{50} = 192 mM) and apomorphin.
Synerazol (137) was isolated from *A. fumigatus* SANK 10588 by the Ando group. The absolute configuration was established by spectroscopic analysis and the modified Mosher’s method. Natural product 137 possesses IgE production inhibitory activity ($IC_{50} = 0.26$ mM; details are given in Section 3-4-2). The fluorinated derivatives showed antiangiogenic activity in a CAM assay and exhibited inhibitory activity against neovascularization. Notably, 19-fluorosynerazol possessed more potent cytotoxicity for several cancer cell lines than natural compound 137.

Cephalimysins A–C (138–140) were isolated from a culture broth of *A. fumigatus* from the marine fish *Mugil cephalus* by Yamada group and FD-838 (141) was isolated from *A. fumigatus* Fresenius F-838 by Omura group. These compounds, except cephalimysin A (138), are structurally similar, differing only in the stereochemistry at the aminal and contiguous hydroxy group. The biological activities of those compounds were also similar, and they showed cytotoxicity against murine P388 and human HL-60 leukemia cell lines. In addition, FD-838 (141) suppressed the growth of certain Gram-positive bacteria and fungi.

Berkeleyamide D (142) was isolated from the fungus *Penicillium rubrum* Stoll by Stierle et al. and inhibited matrix metalloproteinase-3 and caspase-1.

Two tricyclic natural products that have the same oxidation state as these azaspiro products and have a characteristic tricyclic framework incorporating a central $\gamma$-lactam structure, and hemiaminal and acetal functionalities have also been identified. Rubrobramide (143), isolated from *Cladobotryum rubrobrunnescens*, shows antifungal, cytotoxic, and nematicidal activities.
3-1. Hayashi’s Total Syntheses of (−)-Azaspirene, Pseurotin A, and Synerazol

Hayashi’s group achieved the total syntheses of azaspirene (133), pseurotin A (134), and synerazol (137) using convergent strategies with late-stage coupling between a common intermediate and partners bearing different moieties of the target compounds. The common intermediate 151 was synthesized from 2-pentenoate (144) (Scheme 11). Sharpless asymmetric dihydroxylation of 144 was followed by acetonidation to afford acetonide 145. After conversion to ketene silyl acetal from 145, a Mukaiyama aldol reaction promoted by MgBr2·OEt2 against phenylpropargyl aldehyde (146) generated propargyl alcohol 147 with high diastereoselectivity. The protection of the alcohol with a TIPS group and hydration of the ester delivered carboxylic acid 148, followed by amidation with an acid chloride to produce amide 149. Treatment of 149 with NaH under sonication gave lactam 150, which was deprotected at the
acetonide and oxidized at the secondary alcohol to form common intermediate 151. The lithium enolate of 151 was reacted with heptadienal 152 to afford coupling product 153 (Scheme 12). After oxidation, acid-mediated cyclization, and deprotection, the azaspirene (133) was obtained.

Scheme 11. Synthesis of the Common Intermediate 151

Scheme 12. Hayashi’s Total Synthesis of Azaspirene
In the total synthesis of pseurotin A (134), the common intermediate 151 was coupled with known aldehyde 156 to afford lactam 157, and the secondary alcohol was oxidized and cyclized on silica gel during TLC to produce spiro compound 158 (Scheme 13). The selective oxidation of the benzylidene moiety without affecting the other olefin parts was achieved with DMDO and afforded diol 159. Oxidation of benzyl alcohol, desilylation, and aminalization gave pseurotin A (134).

Scheme 13. Hayashi’s Total Synthesis of Pseurotin A

Finally, they reported total synthesis and structural determination of synerazol (Scheme 14). The coupling partner for 151 was synthesized from L-tartaric acid diethyl ester (160). The diol moiety of 160 was converted to an epoxide 161 in two steps. TBS ether 162 was obtained by reducing the ester and installing protecting groups. Transformation of the ester 162 to an aldehyde 163 followed by a Wittig reaction afforded olefin 164, which was deprotected and oxidized to give coupling partner 165. Lactams 151 and 166 were prepared by protecting the secondary alcohol with silyl groups. After coupling with 165 via an aldol reaction, the resultant alcohol was oxidized on silica gel during TLC to form spiro compounds 169 and 170. The hydroxy group on TIPS-protected substrate 171 could not be converted to a methoxy group by the same method as in the pseurotin A synthesis because of acid-mediated decomposition of the epoxide moiety. In contrast, DMDO oxidation in MeOH at the benzylidene moiety on TMS-protected substrate 170 afforded methyl ether 172, although the TIPS-protected substrate produced only diol 171 under the same conditions. Oxidation and deprotection of 172 afforded synerazol (137).
Tadano’s group reported the total syntheses of pseurotin A (134) and F₂ (136), and azaspirene (133).\textsuperscript{70,71} They prepared two segments consisting of a lactam moiety, which was a common intermediate 181, and the side-chain moiety stereoselectively and accomplished the total syntheses via coupling of those compounds. In the total synthesis of pseurotins A and F₂, both segments were synthesized from D-glucose.
Oxidation of known hexafuranose 174 and the addition of a vinyl Grignard reagent provided 175 as a single isomer (Scheme 15). Replacement of the acetonide and chemoselective oxidation with NIS afforded lactone 176, and the lactone moiety was reduced with LiAlH4 to obtain diol 177. The secondary alcohol of 177 was protected with a benzyl group through a sequence involving protection and deprotection of the primary alcohol with a trityl group to afford alcohol 178. After oxidation of primary alcohol, the benzyl Grignard reagent was reacted with aldehyde to afford alcohol 179. In this reaction, using CuBr·Me2S as an additive greatly improved the stereoselectivity. Cyclization was achieved by ozonolysis of the methylene moiety and treatment with TFA, followed by oxidation of hemiacetal to produce lactone 180. Protection of two alcohols with TES, deprotection of the Bn group, and oxidation produced common intermediate 181.

Scheme 15. Synthesis of the Common Intermediate 181 for Pseurotin A and F2 and Azaspirene

In the syntheses of pseurotin A (134) and F2 (136), the diol segment was synthesized from D-glucose (173) (Scheme 16). Alcohol 182, prepared by a known procedure73 from D-glucose (173), was converted to aldehyde 183 by careful protection and deprotection. An aldol reaction between ketone 181 and aldehyde 183 produced adduct 184, which was converted to spiro lactone 185 via deprotection, oxidation, and dehydration. The TES protecting group on the alcohol of lactone 185 was replaced with a MOM group, and the product was treated with NH3 followed by oxidizing the resultant alcohol and
lactamization to afford lactam \textbf{186}. Sequential dehydration, oxidation, and deprotection delivered pseurotin F \textsubscript{2} \textbf{(136)}, which was treated with camphorsulfonic acid (CSA) in MeOH to obtain pseurotin A \textbf{(134)}.

\begin{center}
\textbf{Scheme 16. Tadano’s Total Synthesis of Pseurotin A and F} \textsubscript{2}
\end{center}

In contrast, in the total synthesis of azaspirene, coupling ketone \textbf{181} and (\textit{2E},\textit{4E})-2,4-heptadienal \textbf{(152)} produced \textbf{187} (Scheme 17). The same synthetic route to that of pseurotin A and F \textsubscript{2} was followed up to aminal formation, and then the deprotection of the TES group delivered azaspirene \textbf{(133)}.
3.3. Misaki and Tanabe’s Total Synthesis of Azaspirene

Misaki and Tanabe’s group accomplished the asymmetric total synthesis of azaspirene by using the Ti-Claisen condensation and Ti-direct aldol reaction as key reactions (Scheme 18). First, the chiral template for the Ti-Claisen condensation was prepared. The reaction of tert-butyl acetoacetate (189) with cinnamyl chloride (190) afforded ester 191, which was brominated and deacetylated to give α-bromo ester 192. The tert-butyl moiety of ester 192 was converted into the acid chloride over two steps, and then condensation and cyclization with (R)-atrolanic acid (193) produced 1,4-dioxane derivative 194. The TiCl₄/s-Bu₂NH/2-ethyl-1-methylimidazole-mediated asymmetric Ti-crossed-Claisen condensation between chiral template 194 and propanoyl chloride (195) afforded ester 196, followed by methanolysis to furnish ester 197. Diastereoselective reduction of the ketone moiety of ester 197 with NaBH₄ and ZnCl₂ and acetonidation of the resultant diol produced acetonide 198. After ester hydrolysis, the resultant carboxylic acid was subjected to iodolactonization, and then treated with DBU in the presence of small amount of water to afford lactone-opened carboxylic acid 200. The lactone 201 was prepared by treating carboxylic acid 200 with Ac₂O. Lactam 202 was obtained after oxidation of the vinyl position with SeO₂, treatment with NH₃, and dehydration with acid. Lactam 202 was reduced with NaBH(OAc)₃ with high diastereoselectivity, and the resultant secondary alcohol was protected with an α-chloro-acetyl group. Treatment with TFA produced the diol 204 by acetonide deprotection and caused unexpected migration of the α-chloro-acetyl group. After protection and deprotection, oxidation of the secondary alcohol was followed by the protection of the tertiary alcohol with TMS by using N,O-bis(trimethylsilyl)acetamide (BSA) to produce ketone 205, which was subjected to the TiCl₄/Bu₃N-mediated aldol reaction with (2E,4E)-heptadienal (152) to yield aldol adduct 206. Deprotection of the TMS group, oxidation of the

Scheme 17. Tadano’s Total Synthesis of Azaspirene
secondary alcohol, and treatment with acid formed spiro compound 207, and then the TBS group was removed to afford azaspirene (133).

Scheme 18. Misaki and Tanabe’s Total Synthesis of Azaspirene
3-4-1. Ishikawa’s Synthesis of Synerazol and Pseurotin E

The Ishikawa group reported the one-pot syntheses of synerazol (137) and pseurotin E (135) from pseurotin A (134) and determined the absolute stereochemistry of pseurotin E (Scheme 19). Pseurotin A (134) was obtained from the fermentation broth of *Aspergillus* sp. The Mitsunobu reaction of 134 gave synerazol (137), and selective tosylation in the presence of dibutyltin oxide was followed by epoxidation under basic conditions to afford 208, in which the epoxide moiety was stereoinverted compared with synerazol. Lastly, olefin cross-metathesis using the second-generation Grubbs catalyst with 134 and methyl vinyl ketone produced pseurotin E (135).

Scheme 19. Ishikawa’s Total Synthesis of Synerazol and Pseurotin E

3-4-2. Ishikawa’s SAR Studies of Pseurotin A

The Ishikawa group was interested in the inhibitory activity of pseurotin A (133) against IgE production, which is involved in the pathogenesis of atopy, asthma, rhinitis, and dermatitis. They conducted SAR studies by synthesizing various derivatives of pseurotin A, including pseurotin E (135), pseurotin F$_2$ (136), and synerazol (137). The known compounds obtained from fermentation or precursor-directed biosynthesis were 12,13-dihydropseurotin A (209), 12,13-dibromopseurotin A (210), 10,11-acetonide 211, aldehyde 212, (10R,11S)-diastereomer of synerazol 208, triacetylpseurotin A (214), tetraacetylpseurotin A (215), 17-dihydropseurotin A (216), and fluorinated pseurotin A (217).
Other compounds were prepared by synthetic derivatization of pseurotin A (134) from fermentation (Scheme 20). Compound 134 was subjected to oxidative cleavage to form aldehyde 212, which was converted to olefins 219–224 through the Witting reaction and amines 225–230 by reductive amination. Next, olefin metathesis, as described in Section 3.4.1, of compound 134 gave olefins 231–236, in which the olefin moiety was hydrogenated to produce saturated side chain compounds 237–240. Various modifications of the hydroxy groups, methoxy group, and lactam moiety were performed to produce compounds 241–244. Deoxygenation via compounds 245 and 247 obtained by selective thioacylation afforded compounds 246 and 248. Finally, epoxidation and modified Simmons–Smith cyclopropanation of compound 134 afforded epoxide 249 and cyclopropane 250.

The evaluation of both known and newly synthesized pseurotin analogues revealed that compounds 133, 137, 227, and 248 exhibited IgE inhibitory activity. In particular, compound 248 showed 50-fold higher activity than natural pseurotin A (133). The SAR study indicated that the epoxide stereochemistry, C12-C13 (Z)-olefin, 11-hydroxy group, spiro skeleton, and benzoyl moiety were important for the inhibitory activity.

The specificity of the four compounds showing potent inhibitory activity was investigated further. The concentrations at which IgE production was inhibited were much lower than those at which K562 cytotoxicity was observed or B-cell viability was affected. Furthermore, the most potent inhibitory compound (248) did not inhibit phytohemagglutinin-induced T-cell proliferation (10 μM). Compound 248 also possessed high IgE selectivity (IgM/IgE: 44, IgG2a/IgE: 23, IgG1/IgE: 3.5), whereas the positive control, prednisolone, which is a corticosteroid, showed no selectivity (IgM/IgE: 0.4, IgG2a/IgE: 0.8, IgG1/IgE: 1.0). These data suggest that 248 may be a potent, specific inhibitor of IgE production.
Scheme 20. Ishikawa’s Chemical Modification of Pseurotin A
Table 4. IgE Inhibitory Activity of Pseurotin Analogs

<table>
<thead>
<tr>
<th>Compound</th>
<th>IgE production, IC$_{50}$ (μM)</th>
<th>Compound</th>
<th>IgE production, IC$_{50}$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>133</td>
<td>3.6</td>
<td>229</td>
<td>&gt;10</td>
</tr>
<tr>
<td>135</td>
<td>&gt;10</td>
<td>230</td>
<td>&gt;10</td>
</tr>
<tr>
<td>136</td>
<td>&gt;10</td>
<td>231-236</td>
<td>&gt;10</td>
</tr>
<tr>
<td>137</td>
<td>0.26</td>
<td>237-240</td>
<td>&gt;10</td>
</tr>
<tr>
<td>208-213</td>
<td>&gt;10</td>
<td>241</td>
<td>&gt;10</td>
</tr>
<tr>
<td>214,215</td>
<td>&gt;10</td>
<td>242</td>
<td>&gt;10</td>
</tr>
<tr>
<td>216</td>
<td>&gt;10</td>
<td>243</td>
<td>&gt;10</td>
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<tr>
<td>217,218</td>
<td>&gt;10</td>
<td>244</td>
<td>&gt;10</td>
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<td>219-224</td>
<td>&gt;10</td>
<td>246</td>
<td>&gt;10</td>
</tr>
<tr>
<td>225</td>
<td>&gt;10</td>
<td>248</td>
<td>0.066</td>
</tr>
<tr>
<td>226</td>
<td>&gt;10</td>
<td>249</td>
<td>&gt;10</td>
</tr>
<tr>
<td>227</td>
<td>3.1</td>
<td>250</td>
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<tr>
<td>228</td>
<td>&gt;10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Specificity of IgE Production Inhibitors

<table>
<thead>
<tr>
<th>Compound</th>
<th>IgE production, IC$_{50}$ (μM)</th>
<th>K562 cell cytotoxicity, IC$_{50}$ (μM)</th>
<th>B-cell viability, IC$_{50}$ (μM)</th>
<th>T cell proliferation, % inhibition at 10 μM</th>
<th>MLR$^b$, IC$_{50}$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>133</td>
<td>3.6</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>0%</td>
<td>&gt;10</td>
</tr>
<tr>
<td>137</td>
<td>0.26</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>54%</td>
<td>3.1</td>
</tr>
<tr>
<td>227</td>
<td>3.1</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>2%</td>
<td>nt$^a$</td>
</tr>
<tr>
<td>248</td>
<td>0.066</td>
<td>&gt;10</td>
<td>4.4</td>
<td>0%</td>
<td>nt$^a$</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>0.0059</td>
<td>nt$^a$</td>
<td>&gt;10</td>
<td>84%</td>
<td>0.078</td>
</tr>
</tbody>
</table>

$^a$ Not tested.

$^b$ MLR: Mixed lymphocyte reaction
3-5. Rovis’ Syntheses of Three Diastereomers of Cephalimysin A

Rovis’ group attempted total syntheses of three isomers of cephalimysin A (138) by constructing a spiro ring system with a photoisomerization-coupled asymmetric Stetter reaction using their N-heterocyclic carbenes (254)\(^7\) (Scheme 21). The syntheses began with preparation of substrate for the Stetter reaction in two steps from known bromomaleimide 251\(^78\) with the potassium salt of dialdehyde 252. Although they obtained undesired olefin isomer 253, the problem was solved by \textit{in situ} olefin photoisomerization to the desired isomer. Aldehyde 253 was isomerized under photoirradiation and reacted with known NHC catalyst 254\(^79\) to form spiro compound 255 with high enantioselectivity. The conjugate addition of Grignard reagent 256 in the presence of CuBr to spiro compound 255 generated the enolate intermediate, which was trapped by TBSCl to produce silyl enolate 257. Silyl enolate 257 was treated with benzyl bromide and SmI\(_2\), followed by dehydration with acid to afford benzyldiene-lactam 258. Oxidation of the silyl enol ether and deprotection of the SEM group delivered lactam 259, and the benzyldiene moiety was oxidized by DMDO to afford diol 260. Treatment with Martin’s sulfurane dehydrated the \textit{tert}-alcohol and unexpectedly oxidized the secondary alcohol to produce enone 261. The condition-controlled stereoselective haloetherification yielded products 262 and 263 with opposite stereoselectivity. These compounds were treated with a radical to obtain compounds 264, 265 and 266, respectively. Although they did not obtain cephalimysin A, these synthetic studies provide important knowledge about spiro compound reactivity and spectroscopic data.
Scheme 21. Rovis’s Synthesis of Three Diastereomers of Cephalimysin A
3-6. Švenda’s Total Synthesis of Cephalimysin C

The Švenda group reported the enantioselective total synthesis of both enantiomers of cephalimysin C and B via enantioselective Ni(II)–diamine-catalyzed conjugate addition (Scheme 22).\textsuperscript{80,81} Toward the asymmetric synthesis, they initially performed a racemic synthesis. Titanium enolate generated from readily available α-diazo trifluoroethyl ester \textsuperscript{267} was reacted with commercially available 5-ethylfurfural (\textsuperscript{268}) to produce aldol adduct \textsuperscript{269}, in which oxidation of the resultant alcohol was followed by Rh-catalyzed transformation\textsuperscript{81} to furanone \textsuperscript{270}. Conjugate addition of furanone \textsuperscript{270} to alkynyl ketone \textsuperscript{271} gave enone \textsuperscript{272}, and then Sharpless dihydroxylation of the resultant olefin resulted in spontaneous lactonization and afforded two lactones \textsuperscript{273} and isomer from the diastereomers obtained by dihydroxylation. Desired lactone \textsuperscript{273} was protected with a TMS group and amide \textsuperscript{274} was obtained by ring-opening amidation. The secondary alcohol was oxidized with concomitant heminaminal formation to furnish lactam \textsuperscript{275}. Finally, the deprotection of the TMS group and formation of aminal with a gold catalyst gave (±)-cephalimysin B (\textsuperscript{139}) and C (\textsuperscript{140}). Next, to achieve the asymmetric total synthesis, they performed an asymmetric conjugate addition reaction by using the Ni(II)–diamine complex described by Evans and Seidel.\textsuperscript{82,83} Their optimized conditions using Ni catalyst \textsuperscript{276} afforded the desired product with high enantioselectivity. Because it is easy to prepare both enantiomers of the diamine ligand, conjugate addition could be performed enantioselectively as desired. After the six-step sequence described above, the total syntheses of cephalimysin C, ent-cephalimysin C, and cephalimysin B were accomplished.
Scheme 22. Švenda’s Total Synthesis of Cephalimysin B and C
3-7. Xiao and Wang’s Synthesis of the Spirofuranone-γ-Lactam Core of Cephalimysin A

The Xiao and Wang group reported the stereoselective construction of the spirofuranone-γ-lactam core for the total synthesis of cephalimysin A (138) (Scheme 23).\(^4\) Methyl propionate (277) and known ester 278\(^5\) were subjected to the Claisen condensation, followed by ketal protection to afford ester 279, which was converted to aldehyde 280 via reduction and oxidation. The aldol reaction of aldehyde 280 with the dianion generated from phthalimide 281 produced diol, in which one alcohol was selectively protected with TBS, and oxidation of the other alcohol afforded ketone 282. Oxidation of the methylene moiety with MMPP delivered alcohol 283. After removal of the TBS group, products were separated by silica gel column chromatography, and reprotction of the alcohol with a TBS group was followed by the construction of the spiro skeleton under acidic conditions to give spirofuranone-γ-lactam 284. Although various methods were attempted to install the benzoyl moiety, this was not achieved.

Scheme 23. Xiao and Wang’s Synthetic Studies of Cephalimysin A

3-8. Kuramochi and Tsubaki’s First- and Second-Generation Total Syntheses of Berkeleyamide D and Rubrobramide

Kuramochi and Tsubaki’s group determined the absolute configuration of berkeleyamide D via a short synthesis using their Darzens reaction of α-bromo-β-ketoamide with glyoxals (Scheme 24).\(^6\) The
Darzens reaction of α-bromo-β-ketoamide 285 and isobutyl glyoxal 286 in the presence of a base formed α,β-epoxy-γ-lactam 287 as an intermediate for berkeleyamide D without forming the undesired isomer. The utility of this reaction is also demonstrated in the synthesis of epolactaene with glyoxal. The hemiaminal of lactam 287 was treated with isopropanol and an acid catalyst to produce aminal 288 as a single diastereomer. The ketone in aminal 288 was lithiated and reacted with phenylacetyl chloride (289) to afford spiro compound 291 together with dimer 290 in low yield. Hydrolysis of spiro compound 291 gave (±)-berkeleyamide (142), which was separated by chiral HPLC into (+)-142 and (−)-142, and the absolute configuration was determined by vibrational circular dichroism (VCD).

Scheme 24. Kuramochi and Tsubaki’s First-Generation Total Synthesis of Berkeleyamide D

In 2016, Kuramochi and Tsubaki’s group improved the total yield of berkeleyamide D synthesis by introducing a synthon equivalent to phenylacetyl chloride, used in the previous total synthesis, to the starting material to avoid the problematic acylation (Scheme 25). Carboxylic acid 292 was prepared according to a known procedure from methyl 3-oxo-4-phenylbutyrate and condensation with Meldrum’s acid (293) afforded ester 294. Amidation of ester 294 with hexamethyldisilazane (HMDS) generated amide 295, which was reacted with NBS in the presence of NaHSO₄ to afford bromide 296. The Darzens reaction with bromide 296 and isobutyl glyoxal (286) followed by treatment under acidic conditions completed the modified total synthesis of berkeleyamide D (142).
Scheme 25. Kuramochi and Tsubaki’s Second-Generation Total Synthesis of Berkeleyamide D

Around the same time, they also reported the bioinspired synthesis of rubrobramide (143) based on their γ-lactam construction method with the Darzens reaction and absolute configuration determination by VCD analysis (Scheme 26). Known ketone 298 was protected with an acetal using the Noyori method to afford 1,3-dioxolane 300. The reaction of 300 with HMDS was followed by brominating the methylene with NBS to give substrate 302 for the Darzens reaction. In the presence of triethylamine, 302 and isobutyl glyoxal (286) were reacted to produce tautomers 303/304, which were used in the next reaction without purification because of their instability. 303/304 were treated with acid in CH₂Cl₂ to obtain rubrobramide (143). Esterification with (−)-camphanic chloride (305) followed by separation of the diastereomers and methanolysis afforded optically pure (−) and (+)-rubrobramide (143).
3-9. Han’s Total Syntheses of Berkeleyamide D, Azaspirene, FD-838, and Cephalimysin A

Han’s group reported the total syntheses of berkeleyamide D (142),92 azaspirene (133),93 FD-838 (141),94 and cephalimysin A (138)94 based on a strategy inspired by the biosynthetic origin of azaspirene95 and the biomimetic synthesis of α,β-unsaturated γ-hydroxy-γ-lactam and α,β-epoxy-γ-hydroxy-γ-lactam ring systems reported by the Snider group.96 Initially, they achieved the total synthesis of berkelyamide D (142) (Scheme 27).92 The reaction of dienolate generated from tert-butyl acetoacetate 189 and phenylacetaldehyde 307 produced aldol adduct 308, in which the secondary alcohol was protected with a TBS group and the tert-butyl ester was condensed with L-leucinol (307) to afford amide 310. Oxidation of alcohol 310 under modified Pfitzner-Moffatt conditions was followed by treatment with base to give lactam 311, although the asymmetric center was lost. In this cyclization, when amide 312, prepared from 308 in four steps, was used as a substrate, the undesired cyclization proceeded to give 7-membered product 313. Continuous oxidation, consisting of electrophilic epoxidation followed by nucleophilic epoxidation, of lactam 311 to α,β-epoxy-γ-hydroxy-γ-lactam 315 was performed using magnesium
monoperoxyphthalate (MMPP). Subsequently, desilylation of the secondary alcohol and hydroxy to methoxy substitution of 315 by treatment with CSA in methanol provided alcohol 316. Oxidation of the secondary alcohol followed by treatment with a base generated spiro compound 317, which was subjected to acidic conditions to produce berkeleyamide D (142).

Scheme 27. Han’s Total Synthesis of Berkeleyamide D

Han’s group used the same synthetic method\textsuperscript{92} to achieve the total synthesis of azaspirene from β-ketoester 319, prepared from α,β,γ,δ-unsaturated aldehyde 152 and β-ketoester 318 (Scheme 28).\textsuperscript{93} Oxidation of the secondary alcohol of 319 was followed by condensation with L-phenylalaninol (320) to produce amide 321. Amide 321 was subjected to a modified Pfützer–Moffatt oxidation and treatment with base to afford lactam 322. Interestingly, the cyclization of β,δ-diketoamide 321 produced the γ-lactam, although the reaction of β,δ-diketoamide 312 gave the 7-membered lactam (Scheme 27). Because the methyl group at the γ-position in substrate 321 precluded the dehydration reaction that
formed the 7-membered lactam, the reactions of β-ketoamide 312 or β-ketoamide 321 proceeded differently. After continuous oxidation using MMPP, spontaneous spiro cyclization delivered (±)-azaspirene (133).

Han’s group then attempted the asymmetric syntheses of berkeleyamide D and azaspirene by using an asymmetric epoxidation as part of the continuous oxidation, but this approach did not work. Finally, they achieved the total syntheses of FD-838 (141) and cephalimysin A (138) by chiral pool synthesis (Scheme 29). These total syntheses used β-ketoester 318, the appropriate aldehyde 268 or 324, and amine 327, which contains two chiral centers. Spiro compounds consisting of an inseparable mixture of 330 and 331 or 332 were obtained in same manner as the previous azaspirene synthesis. Treatment of spiro compounds 330 and 332 with camphorsulfonic acid in methanol produced only methoxy derivatives 333.
or 334 as single diastereomer. After deprotection of TBS group and oxidation of the resultant alcohol, the total syntheses of FD-838 (141) and cephalimysin A (138) were completed.

Scheme 29. Han’s Total Syntheses of Cephalimysin A and FD-838
Kanomata and Emoto’s Synthesis of the Azaspirene Skeleton

Kanomata and Emoto synthesized the azaspirene skeleton to elucidate its racemization (Scheme 30). The synthesis used a convergent approach with a lactam segment bearing an isocyanate group and a furanone segment. Commercially available α-acetamidocinnamic acid (335) was converted to ester 336, and the acetyl group on the amine was replaced with a Boc group to afford ester 337. The Kokotos protocol was used to react the ester with trifluoromethanesulfonic anhydride and 2-chloropyridine as base to give isocyanate 338. Next, the furanone segment was synthesized and used to complete the synthesis of the azaspirene skeleton. Claisen condensation between 3-pentanone (339) and benzyloxyacetyl chloride (340) gave 1,3 diketone 341, followed by hydrogenolysis of the benzyl group and spontaneous lactonization to produce furanone 342. The anion generated from furanone 342 was reacted with isocyanate 338, and then the resultant product was protected with a MOM group to afford furan 343. The ester was converted to aldehyde 344 by reduction and oxidation, and then treatment with aqueous hydrochloric acid in DMSO produced diastereomers 345–347, which were neutralized with aqueous NaOH solution under heating to afford thermodynamically favored 345. Diastereomeric resolution by Shiina’s method which involves installation of a serine derivative and separation, and subsequent hydrolysis afforded enantiomerically pure azaspirene derivatives (+)-345 and (−)-345. Emoto’s group reported that compounds (+)-345 and (−)-345 have antiangiogenic activity and can inhibit the growth of human uterine carcinosarcoma.
4. CONCLUSION

In this review, we have discussed the synthesis and biological studies of highly oxidized $\gamma$-lactam-containing natural products and related molecules, focusing on epolactaene; NG-391, lucilactaene; L-755,807; azaspirene; pseurotins A, E, and F$_2$; synerazol; cephalimysins A-C; FD-838; berkeleyamide D; rubrobramide; and talaramide A. Their complexity and densely packed functional groups allow for a diverse array of synthetic approaches, and many researchers have worked to establish elegant and efficient synthetic routes. Moreover, the wide-ranging biological activities of these molecules, which likely arise from the high reactivity of their highly oxidized multifunctional structures, have
attracted much attention in medical research. These molecules will continue to serve as attractive synthetic and pharmaceutical targets in drug discovery.

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REFERENCES
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